Received: 5 October 2016 | Accepted: 22 December 2016

DOI: 10.1111/ina.12366

ORIGINAL ARTICLE

WILEY

Health effects of laser printer emissions: a controlled exposure study

S. Karrasch[1,2] | M. Simon[1] | B. Herbig[1] | J. Langner[3] | S. Seeger[3] | A. Kronseder[1] | S. Peters[1] | G. Dietrich-Gümperlein[1] | R. Schierl[1] | D. Nowak[1,4] | R. A. Jörres[1,4]

[1]Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Inner City Clinic, University Hospital of Munich, Ludwig-Maximilians-Universität, Munich, Germany

[2]Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany

[3]Division Materials and Air Pollutants, BAM Federal Institute for Materials Research and Testing, Berlin, Germany

[4]Comprehensive Pneumology Center Munich (CPC-M), Member of the German Center for Lung Research, Munich/Neuherberg, Germany

Correspondence
Dr. Stefan Karrasch, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Inner City Clinic, University Hospital of Munich, Ludwig-Maximilians-Universität, Munich, Germany.
Email: stefan.karrasch@med.uni-muenchen.de

Funding information
German Statutory Accident Insurance, Grant/Award Number: FP294

Abstract

Ultrafine particles emitted from laser printers are suspected to elicit adverse health effects. We performed 75-minute exposures to emissions of laser printing devices (LPDs) in a standardized, randomized, cross-over manner in 23 healthy subjects, 14 mild, stable asthmatics, and 15 persons reporting symptoms associated with LPD emissions. Low-level exposures (LLE) ranged at the particle background (3000 cm^{-3}) and high-level exposures (HLE) at 100 000 cm^{-3}. Examinations before and after exposures included spirometry, body plethysmography, transfer factors for CO and NO (TLCO, TLNO), bronchial and alveolar NO, cytokines in serum and nasal secretions (IL-1β, IL-5, IL-6, IL-8, GM-CSF, IFNγ, TNFα), serum ECP, and IgE. Across all participants, no statistically significant changes occurred for lung mechanics and NO. There was a decrease in volume-related TLNO that was more pronounced in HLE, but the difference to LLE was not significant. ECP and IgE increased in the same way after exposures. Nasal IL-6 showed a higher increase after LLE. There was no coherent pattern regarding the responses in the participant subgroups or single sets of variables. In conclusion, the experimental acute responses to short but very high-level LPD exposures were small and did not indicate clinically relevant effects compared to low particle number concentrations.

KEYWORDS
emission, exposure, laser printer, lung function, respiratory health, ultrafine particles

1 | INTRODUCTION

Laser printing devices (LPDs) are ubiquitous and a source of indoor air pollution.[1–5] They typically emit particles below 300 nm in diameter in varying quantities.[5] As emission levels vary substantially between devices, categorizations based on the particle outputs ranging from non-emitter to high-level emitter have been proposed.[6] In principle, the emissions may be harmful to human health, as might be suspected from the effects of indoor and outdoor aerosols from other sources which are well known to elicit adverse effects.[7]

The possible specific health risks of LPD emissions have attracted attention for many years,[8–11] and there are several case reports on health effects[12–15] as well as studies investigating groups of exposed persons.[16,17] Similar to the case reports, these studies showed associations but are not conclusive regarding causality, mostly due to uncontrolled confounders or the difficulty to define appropriate control groups. Unfortunately, large-scale epidemiological data on health effects of LPD exposure are lacking. Those on toner exposure[18–20] are not helpful for the present question as the particles emitted from LPDs are clearly different from original toner particles.[21,22]

Potential adverse effects of LPD emissions have also been suggested by the results of animal[23] and cell culture experiments.[24] However, these data are difficult to extrapolate to human exposure conditions despite attempts to match the doses of particles. The only quasi-experimental human exposure study published until now involved a small group of subjects exposed to lower particle

concentrations and a smaller number of parameters than used here. On the other hand, the time of exposure was longer.[16]

We followed a controlled, cross-over experimental design to investigate the effects of LPD particle emissions *in vivo* in human subjects, using either high-level (HLE) or low-level (LLE) exposures. The study included 23 healthy volunteers, 14 with mild asthma, and 15 who reported complaints from previous LPD exposure. The measurements comprised a broad panel of physiological and biochemical assessments focusing on acute effects.

Practical Implications

- This study investigated acute effects of an exposure to very high levels of LPD emissions versus a low particle background exposure and included participants who might be regarded as particularly susceptible. Although a broad panel of sensitive outcome measures was used, the results after a single high-level exposure to LPD emissions did not indicate alterations that would correspond to the spectrum of suspected health impairments. These findings suggest that acute complaints reported after exposure to LPD emissions are probably not based on objective, clinically relevant changes and that other causes have to be considered.

2 | MATERIALS AND METHODS

2.1 | Setup and study design

The experiments were performed under standardized conditions in an exposure chamber (volume 32 m^3) at the Ludwig-Maximilians-Universität (LMU), Munich. The chamber was unventilated during the exposures. An overview of the chamber setup is shown in Figure 1. Participants were studied in low-level and high-level exposure sessions in a single-blinded manner on two different days in random order, and only one participant per session. HLE were generated by two high-emitting laser printers (HE) operated alternatingly within a time span of 75 minutes of which the last 60 minutes were kept at a constant exposure level. In the same way, two low emitters (LE) were operated in the LLE sessions; their particle emissions did not measurably increase the background number and mass concentrations. The total time which the participants spent in the chamber was about 105 minutes. They were blinded to the type of exposure but could recognize when the LPDs were active. During each printing session, the particle size distribution as well as the number and mass concentrations were continuously monitored by aerosol spectrometers covering a size range from 5.6 nm up to 20 μm (Fast Particle Sizers EEPS and FMPS; TSI Inc., Shoreview, MN, USA; Optical Particle Counter OPC 1108; Grimm Aerosol Technik GmbH & Co. KG, Ainring, Germany). As a control for the reproducibility of exposures, an exposure parameter

$$E = \frac{\int C(t)\mathrm{d}t}{T} \quad (1)$$

was defined, with the total particle number concentration at time t, $C(t)$, and the participant's total time in the chamber, T; the exposure parameter was also computed for the final 60 minutes of exposure.

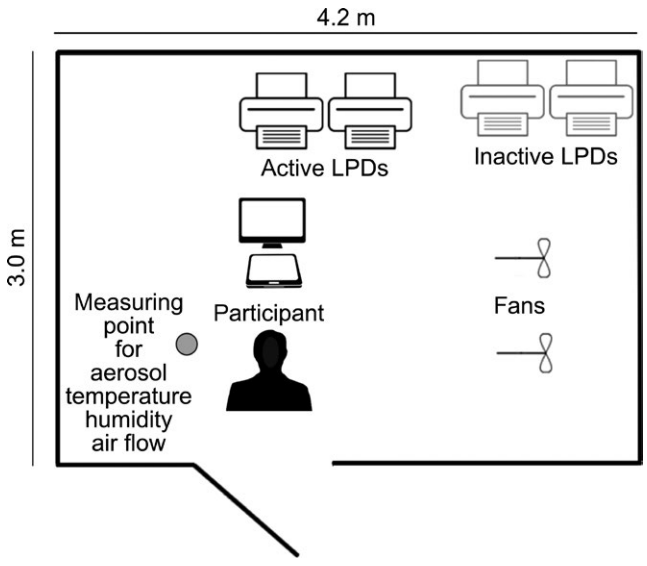

FIGURE 1 Exposure chamber setup with positions of the participant, the measuring point, and the active and inactive laser printing devices (LPDs)

We considered a total exposure period of 75 minutes duration as adequate to provoke effects during exposure sessions. The functional and biochemical assessments were performed in the same order before and after exposures, and they were chosen to address as many as possible of the effects typically reported by persons with self-reported symptoms. One sequence of measurements took about 2 h, which resulted in a total duration of about 5.5 h for each exposure session (for details, see Table 1). Upon enrollment and prior to the exposures, all participants were characterized in an initial examination at which they also were trained in performing the assessments.

To account for possible circadian effects, all exposure days started at 10.00 AM, thereby ensuring that differential effects were to be attributed solely to the exposures. During exposures, participants were sitting at a desk in an office-like situation. This position was also required for performing neurocognitive tests that were also part of the study. These results are not covered in the present manuscript as they represent a separate, large data set.

2.2 | Study participants

Participants were recruited from the LMU Outpatient Clinic for Occupational, Social and Environmental Medicine, by notices and intranet announcements, by an information letter to pneumologic practices and to the Munich municipality, as well as via local media. Volunteers of age 18-60 y were included. Exclusion criteria comprised significant impairments of health, such as diabetes mellitus and cardiac

TABLE 1 Sequence of assessments on exposure days (results of questionnaires and neurocognitive tests are not part of the present evaluation)

"pre"-examination	Exhaled CO
	FeNO and CANO
	Transfer factor for CO and NO
	Spirometry and body plethysmography
	Exhaled breath condensate
	Blood samples
	Nasal secretions
	(questionnaires)
	Exposure chamber entry
	(neurocognitive tests)
	Exposure
"post"-examination	*(neurocognitive tests)*
	Exposure chamber exit
	(questionnaires)
	Exhaled CO
	FeNO and CANO
	Transfer factor for CO and NO
	Spirometry and body plethysmography
	Exhaled breath condensate
	Blood samples
	Nasal secretions

diseases, as well as current smoking and the use of corticosteroids 3 weeks prior to the initial examination or during the study.

The participants recruited were either persons with self-reported symptoms associated with exposure to LPD (S), or with mild stable asthma without self-reported LPD-related symptoms (A), or healthy controls without these characteristics (H). LPD-related symptoms could comprise dyspnea, cough, ocular irritation, fatigue, dizziness, or other symptoms commonly reported. For asthma, a diagnosis by a physician was required. Across these groups, participants were also evaluated regarding the presence of bronchial hyperreactivity (BHR) which was assessed using a methacholine inhalation challenge at the initial examination.

The study was approved by the Ethical Review Committee of the LMU University Hospital, and written informed consent was obtained from all participants.

2.3 | Assessments

2.3.1 | Spirometry and body plethysmography

Spirometry and body plethysmography were performed according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines[25] and the recommendations of the German Society for Pneumology (DGP)[26] using a Bodyscreen device (Jaeger-CareFusion, Höchberg, Germany). Intrathoracic gas volume (ITGV) was determined from at least two acceptable shutter maneuvers, and airway resistance (Raw) and specific airway resistance (sRaw) were based on five reproducible resistance loops. For spirometric parameters, three acceptable and reproducible maneuvers were required. Spirometry was performed after determination of airway resistance and ITGV.

2.3.2 | Bronchial challenge test

At the initial examination, all participants were tested for bronchial hyperreactivity (BHR) using a methacholine (MCh) inhalation challenge (3.2% MCh solution, five dose increments) in a modified dosimeter method (Jaeger-CareFusion, Höchberg, Germany).[27] BHR was assumed if sRaw doubled and simultaneously increased above 2 kPa×s after inhalation of the maximal dose or a lower dose.

2.3.3 | Transfer factors of the lung for nitric oxide (NO) and carbon monoxide (CO)

To assess potential effects of LPD emissions on gas exchange, the transfer factor of the lung for CO (TLCO) was determined by the single-breath method following the ERS guidelines[28] and using a Masterscreen PFT instrument (Jaeger-CareFusion, Höchberg, Germany). In the same maneuver, the transfer factor for NO (TLNO) was measured. This parameter is considered to be capable of differentiating between effects on diffusion limitation and capillary blood volume.[29] The breath-hold time was set to 8 s, and at least two acceptable and reproducible measurements were required. TLCO results were corrected for hemoglobin (Hb) concentrations measured in the EDTA blood sample as well as for carboxyhemoglobin (COHb) values which were derived from exhaled carbon monoxide (eCO).[30] In some subjects, eCO could not be measured after exposure due to organizational restrictions (n=27 for LLE and n=26 for HLE) and the increase in COHb was estimated as the mean increase in participants with measurements after exposure (5.7±2.3 ppm). Alveolar volume (VA) was determined by helium dilution in the single-breath maneuver, and the transfer coefficients for CO (KCO) and NO (KNO) were computed by dividing TLCO and TLNO, respectively, by VA.

2.3.4 | Exhaled carbon monoxide and exhaled nitric oxide

The value of eCO was used to validate the non-smoking status of the participants and to adjust TLCO results for COHb. After a deep expiration, a subsequent maximal inspiration, and a breath-hold of 10 seconds, participants expired into the sampling tube of the instrument (BreathCO, Vitalograph, North Buckinghamshire, UK). The fractional exhaled nitric oxide (NO) at a flow rate of 50 mL/s (FeNO), as an indicator of bronchial NO production, was measured according to the ATS/ERS guidelines.[31] Additionally, the concentration of alveolar NO (CANO) was estimated from measurements at target flow rates of 158.1, 238.3, and 309.6 mL/s. The values of exhaled NO at these flow rates were used to calculate the value of CANO; this was based on

a linearized version of a nonlinear, two-compartment model describing the NO transfer within the lung.[32] Measurements were performed without nose clips to achieve velum closure. They comprised a maximal inspiration and subsequent expiration into a mouthpiece with defined resistances at a target pressure of 12 mm Hg. The actual pressure was shown on a display to help the subjects in achieving the constant flows. At least three values per flow rate were obtained. NO was measured by a fast chemiluminescence analyzer (NOA280, Sievers, Boulder, Co, USA).

2.3.5 | Hydrogen peroxide in exhaled breath condensate

For the collection of exhaled breath condensate (EBC), participants breathed normally into an EcoScreen device (Jaeger-CareFusion, Höchberg, Germany) for 15 minutes while sitting and wearing nose clips. As a variable fraction of hydrogen peroxide (H_2O_2) in EBC can be attributed to H_2O_2 inhaled from ambient air,[33] an inspiration filter was used that eliminated most (80%) of the inspired H_2O_2. EBC samples were processed immediately after collection and analyzed in duplicates by an optimized fluorimetric method (for details, see Peters et al.[33]).

2.3.6 | Biomarkers in nasal secretions and serum

At the end of each examination, serum samples obtained from venous blood and nasal secretion samples were collected (Table 1) and stored at −20°C until analysis. To obtain nasal samples, cotton rolls were placed in the middle meatus of both sides of the nose for 15 minutes. After removal, secretions were retrieved by centrifugation. Depending on the yield, the procedure was repeated until a sufficient sample volume was achieved. In both serum and nasal samples, interleukin (IL)-1β, IL-5, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFNγ), and tumor necrosis factor alpha (TNFα) were measured using a Bio-Plex Pro Assay (Bio-Rad Laboratories Inc., Hercules, CA, USA). Moreover, in the serum samples, the levels of eosinophilic cationic protein (ECP) and total immunoglobulin E (IgE) were determined (ImmunoCAP, Thermo Fisher Scientific Inc., Waltham, MA, USA), as well as those of 8-hydroxy-desoxy-guanosine (8-OHdG) in triplicates after serum ultrafiltration (Highly Sensitive 8-OHdG ELISA kit, Japan Institute for the Control of Aging, JaICA, Nikken SEIL Co., Ltd. Haruoka, Fukuroi, Shizuoka, Japan).

2.4. | Statistical analysis

For descriptive purposes, medians and interquartile ranges (IQR) are given. To use the parameters in parametric analyses, their distribution was tested for normality according to Kolmogorov-Smirnov and values were log-transformed if appropriate. Moreover, to minimize numeric effects of cytokine values below the limit of detection in parametric analyses, parameter-specific limits of detection were set to be slightly below the lowest measured value.

In a first step, the effects of exposures were evaluated for each emitter condition separately by paired comparisons of the data obtained pre- versus post-exposure. Paired comparisons were also used to compare the pre-post differences between emitter conditions, as well as the values before the two exposures. To achieve the same power in all tests, the nonparametric Wilcoxon matched-pairs signed-ranks test was used for all of these comparisons.

In a second step, potential differences between exposures were studied in a more comprehensive statistical model. For this purpose, two-way repeated-measures analysis of variance (ANOVA) was employed, with the categorical factors pre-post exposure and emitter condition and the interaction between them. To test for differences between the three participant groups or between participants with versus without BHR (assessed by methacholine inhalation challenge), additional repeated-measures ANOVAs were performed including the participant group or BHR as between-subject factor, respectively, as well as interactions which were taken as indicators of differences between groups.

To look for more global patterns of changes, we first condensed the information from three groups of variables (blood-derived parameters, biomarkers from nasal secretions, lung function parameters) via explorative factor analyses with varimax rotation. Each of the analyses used the pre- and post-values of both exposures. The stability of the resulting factorial structure was checked in factor analyses of the pre- and post-values for both exposures separately. Factors with an eigenvalue ≥1 were retained. In a second step, the derived factor scores were calculated and evaluated in the same way as the original measurements.

In all evaluations, missing values were excluded listwise, which resulted in slightly different numbers of data in different tests. Statistical analyses were performed using the software packages SPSS Statistics 23 (IBM Corp., Armonk, NY, USA) and Statgraphics Centurion XVII (Statpoint Technologies, Inc., Warrenton, VA, USA). To avoid the discarding of possibly meaningful effects, results were considered as statistically significant at the level p=0.05 despite the multiplicity of tests. Essentially, there were five comparisons per parameter: pre vs pre, post vs post, two times pre vs post, difference vs difference, which would result in a (conservative) Bonferroni factor of 5.

3 | RESULTS

3.1 | Exposure characteristics

Figure 2 shows the typical particle size distribution in the chamber during a HLE session. For particles with a diameter above 350 nm, concentrations were at or below the detection limits of the instruments. During HLE, the size distribution of ultrafine particles typically developed a slight shift over time as illustrated in Figure 3 by the size spectra obtained 5, 40, and 75 minutes after start of printing. Figure 4 demonstrates the development over time of the total particle number concentrations during HLE and LLE. During HLE, a total number concentration of 10^5 per cm^3 was built up within 15 minutes and kept stable over the remaining 60 minutes. The small peaks indicate the alternating printing action of the two LPDs. During LLE, no contribution from LPD operation was visible.

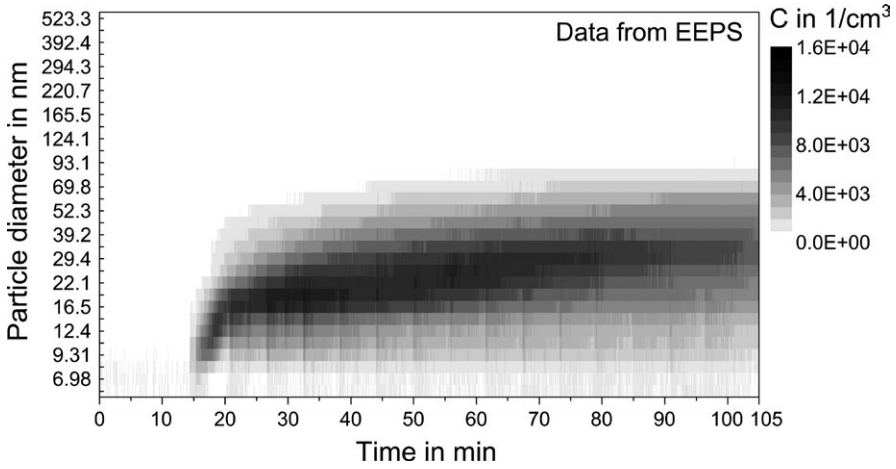

FIGURE 2 Typical particle number size distribution during a high-level exposure (HLE) session in the size range between 5.6 nm and 560 nm

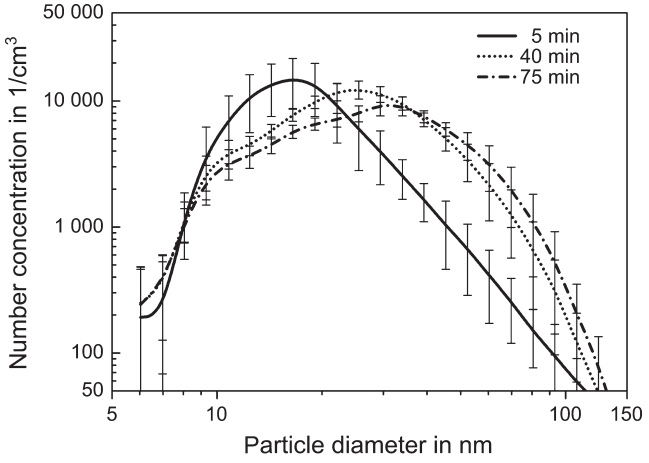

FIGURE 3 Average high-level exposure (HLE) particle number size distributions and standard deviations 5, 40, and 75 minutes after start of printing

The results for *E* referring to the subjects' total chamber time of about 105 minutes are given in Figure 5. With a standard deviation of $\pm 1.1\times10^4$ cm^{-3} (ie, coefficient of variation <12%), the dispersion around $E_{mean}=9.6\times10^4$ cm^{-3} was very low. There were no statistically significant differences in HLE levels between the groups of participants, and during the final 60-minutes plateau, the coefficient of variation was less than 10%.

Figure 6 shows the mean particulate matter (PM) mass concentrations for PM_{10} and $PM_{2.5}$ monitored continuously during the sessions. There was no difference between HLE and LLE. The LLE levels were due to contamination from the outside. An example of the time course of temperature and humidity during exposures is shown in Figure 7. Temperature and humidity changed in a similar manner in all exposures; there were no complaints from participants about these changes.

3.2 | Study participants

Overall, 52 participants were studied: 23 healthy (H), 14 with mild bronchial asthma (A), and 15 reporting symptoms associated with LPD emissions (S). A description of the study population is given in Table 2. The group of asthmatics was significantly younger than the two other groups. The spectrum of symptoms reported after previous LPD exposure in group S is illustrated in Figure 8; symptoms of the lower and upper respiratory tract and eye symptoms were the most frequent ones.

3.3 | Results of exposures

An overview of the effects of exposures on the parameters presented below is given in Table 3.

3.3.1 | Spirometry and body plethysmography

Overall, baseline values before the two exposures did not significantly differ from each other for all lung function parameters, except for a small difference regarding ITGV, with an average difference of 73 mL ($P<.01$). Spirometric and body plethysmographic parameters did not significantly change over HLE or LLE. Moreover, in ANOVA comparisons, no statistically significant effects were detected.

3.3.2 | Transfer factor for CO and NO

The values of TLCO, TLNO, and VA were comparable before the two exposures. In pairwise comparisons taken over all participants, there were significant decreases in TLCO after both LLE and HLE ($\Delta_{LLE}=-2.0\%$, $P<.01$; $\Delta_{HLE}=-2.2\%$, $P<.005$). Similarly, TLNO decreased after both exposures ($\Delta_{LLE}=-1.9\%$, $P<.005$; $\Delta_{HLE}=-2.5\%$, $P<.005$), as well as VA decreased ($\Delta_{LLE}=-0.9\%$, $P<.01$; $\Delta_{HLE}=-0.3\%$, $P<.05$). The transfer coefficients KCO and KNO showed a similar pattern as TLCO and TLNO but only the change in KNO reached statistical significance after both exposures (KCO: $\Delta_{LLE}=-1.0\%$, n.s.; $\Delta_{HLE}=-0.7\%$, n.s.; KNO: $\Delta_{LLE}=-0.8\%$, $P<.05$; $\Delta_{HLE}=-2.2\%$, $P<.005$). None of the pre-post differences in transfer factors or coefficients significantly differed between HLE and LLE. The results of these pairwise comparisons were supported by two-way repeated-measures ANOVA. These analyses confirmed the overall decreases in TLCO, TLNO, KCO, KNO, and VA after both exposures, but without significant interactions with the exposure condition HLE versus LLE.

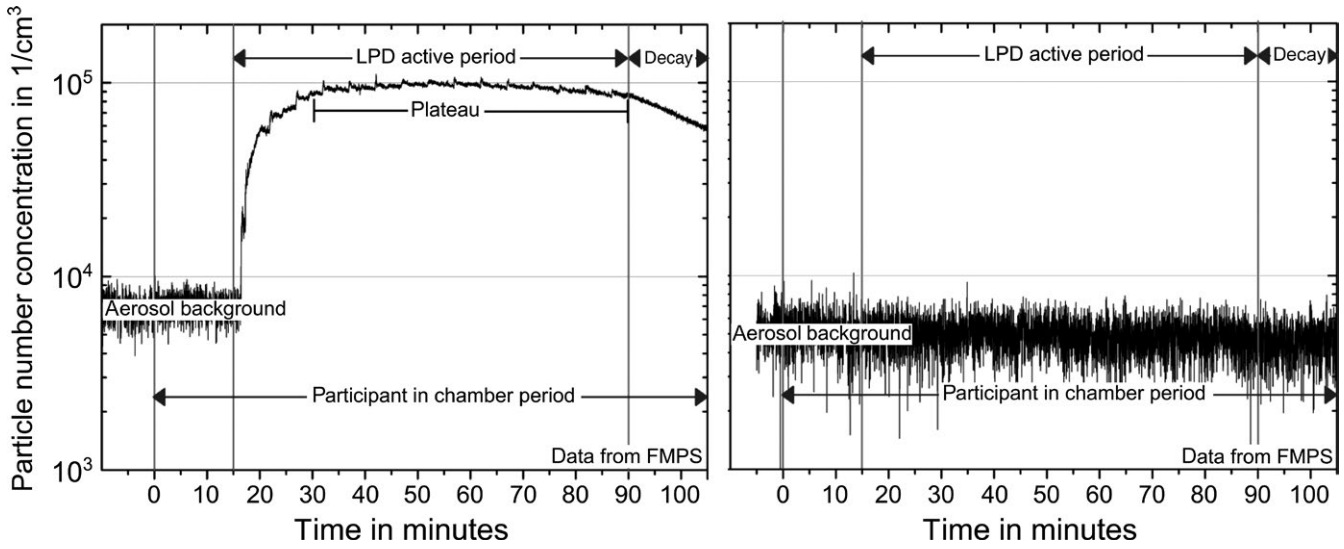

FIGURE 4 Typical total particle number concentrations during a high-level exposure (HLE; left) session and a low-level exposure (LLE; right) session

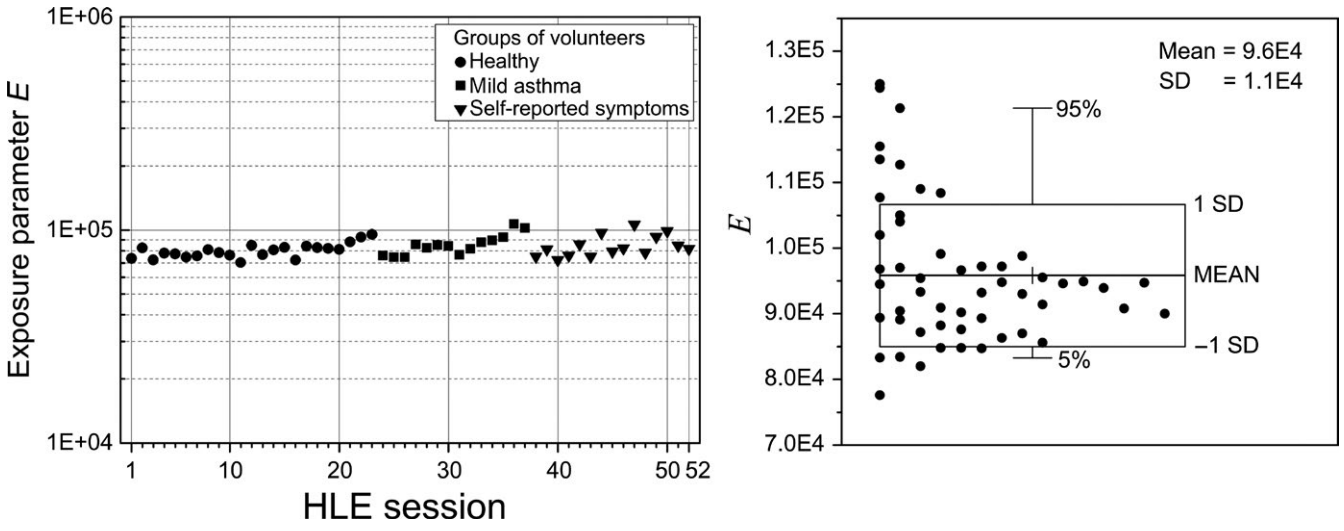

FIGURE 5 Quantitative repeatability of high-level exposure (HLE): Left: exposure parameters for all sessions and participant subgroups. Right: statistical analysis of the exposure parameter *E*

3.3.3 | Exhaled biomarkers

The values of eCO were comparable before HLE and LLE. Those after both exposures (n=24 for LLE and n=26 for HLE) were elevated as the result of the prior TLCO measurements that were accompanied by CO inhalation. The values of FeNO and CANO were also comparable before exposures. There were no statistically significant changes in both parameters, neither after HLE nor after LLE. The values of exhaled H_2O_2 were available only in a subset of participants due to technical difficulties in the measurement of the very low concentrations (22/18 participants for LLE/HLE). Both exposures showed a significant increase in H_2O_2 levels (Δ_{LLE} =41.8%, P<.005; Δ_{HLE} =36.5%, P<.005) but without a significant difference between the changes observed in both exposures.

3.3.4 | Biomarkers in serum and nasal secretions

The serum levels of IL-1β, IL-5, IL-6, IL-8, GM-CSF, IFNγ, and TNFα prior to LLE and HLE did not significantly differ from each other. Particularly, for IL-1β but also for IL-5, a large proportion of values was below the detection limits. For IL-8, a significant decrease was observed during LLE (P<.05), while no effect occurred during HLE; the changes did not significantly differ between both exposures. In the ANOVA, serum IL-6, IL-8, GM-CSF, IFNγ, and TNFα showed a significant decrease during exposures (P<.05) but without a significant effect of or interaction with the emitter condition. Furthermore, the serum levels of 8-OHdG were comparable before exposures and showed no significant changes during or differences between exposures.

Regarding nasal secretions, the levels of the measured interleukins were comparable before LLE and HLE, except for IL-1β, as reflected in

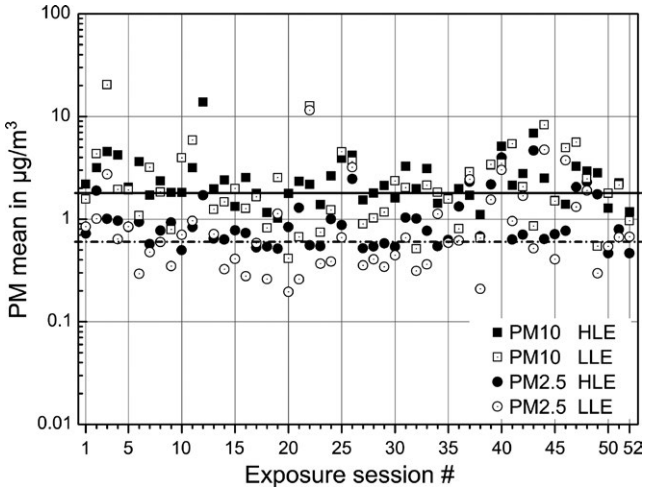

FIGURE 6 $PM_{2.5}$ and PM_{10} session mean values

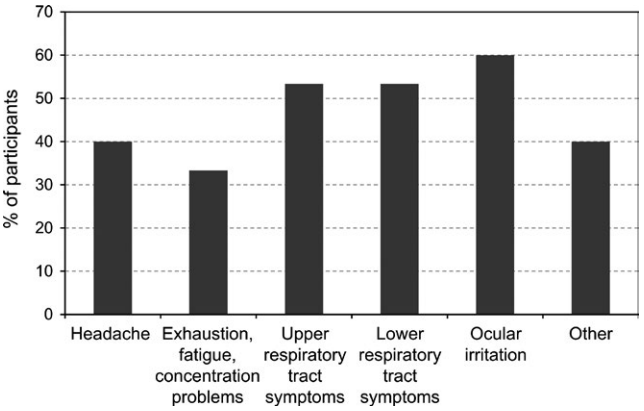

FIGURE 8 Symptoms associated with laser printing device (LPD) exposure reported by the subjects in the participant group with LPD-related complaints

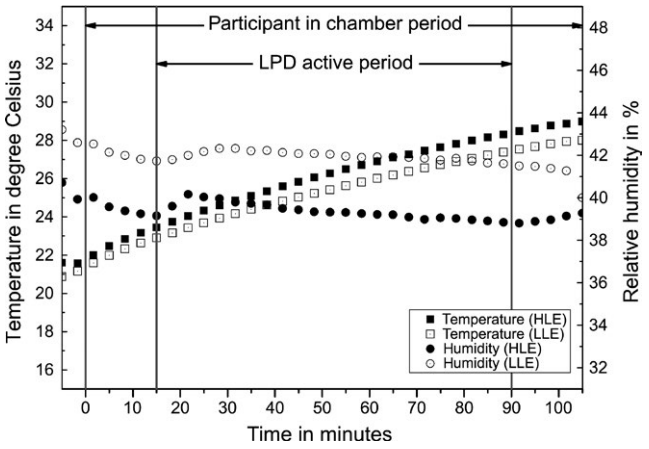

FIGURE 7 Exemplary variations of temperature and humidity in the chamber during an exposure session

a mean difference of 11.5 pg/mL ($P<.05$). In the nasal samples, the proportion of values below the detection limit was low. Statistically significant changes over exposures occurred for IL-6 after both HLE and LLE (Δ_{LLE}=96.6%, $P<.005$; Δ_{HLE}=30.8%, $P<.005$). Here, a significant difference between the changes was observed ($P<.05$; see Table 3); that is, for the LLE, the increase was more pronounced than in the HLE. ECP and IgE values in serum were comparable before the two exposures. ECP levels increased after both LLE and HLE (Δ_{LLE}=12.3%, $P<.01$; Δ_{HLE}=16.4%, $P<.005$); the same was true for IgE (Δ_{LLE}=1.4%, $P<.01$; Δ_{HLE}=1.2%, $P<.05$). For both ECP and IgE, the changes over the two exposures were not significantly different from each other.

3.3.5 | Factor analysis

An overview of the results of the factor analyses is shown in Table 4A-C. When applied to the lung function parameters forced expiratory volume in 1 second (FEV_1), its ratio to forced vital capacity (FEV_1/FVC), ITGV, sRaw, KCO, KNO, three factors resulted. According to the loadings, the first factor comprised KCO and KNO; the second, FEV_1/FVC and sRaw and the third, FEV_1 and ITGV (Table 4a). Only the scores for the first factor showed a significant decrease over HLE ($P<.05$), but again, there was no significant difference between the changes over both exposures, neither in terms of the interaction term nor in the comparison of the pre-post differences (Table 5).

The factor analysis of IL-1β, IL-5, IL-6, IL-8, GM-CSF, IFNγ, and TNFα in nasal secretions resulted in two factors. The first one covered IL-5, GM-CSF, IFNγ, and TNFα, and the second, IL-1β, IL-6, and IL-8. The scores of the second factor were significantly different between pre- and post-values over both exposures ($P<.05$ each). There was no significant difference between exposures, as indicated by the interaction term between pre-post and emitter condition, as well as the comparison of pre-post differences of HLE versus LLE. For the first factor, no statistically significant changes or differences were observed.

TABLE 2 Characteristics of the study population. Means and standard deviations are given if not stated otherwise

	Healthy participants	Participants with asthma	Participants with self-reported symptoms
Participants, n (m/f)	23 (12/11)	14 (5/9)	15 (3/12)
Age, years (min; max)	43.6±12.5 (20; 60)	35.6±11.6 (21; 57)	47.6±6.8 (33; 58)
Height, cm	174.3±5.5	169.4±9.9	167.5±9.8
BMI, kg/m^2	25.0±3.6	24.6±1.8	25.0±4.7

TABLE 3 Overview of changes after low-level exposure (LLE) and high-level exposure (HLE)

Parameter	LLE			HLE			Difference between changes (Wilcoxon test[d]) *P* values	Interaction between changes and emitter condition (ANOVA) *P* values	Log[b]
	Absolute change Median (25%; 75%)	Change in % baseline Median (25%; 75%)	Wilcoxon test[c] *P* values	Absolute change Median (25%; 75%)	Change in % baseline Median (25%; 75%)	Wilcoxon test[c] *P* values			
FEV_1, L	0.01 (−0.05; 0.07)	0.3 (−1.2; 1.8)	.329	0.00 (−0.05; 0.07)	−0.2 (−1.5; 2.1)	.698	.943	.571	
FVC, L	−0.01 (−0.07; 0.03)	−0.3 (−1.7; 0.8)	.170	−0.04 (−0.11; 0.03)	−0.9 (−2.8; 0.7)	.052	.445	.386	
ITGV, L	0.00 (−0.11; 0.10)	0.0 (−3.3; 3.4)	.866	−0.04 (−0.12; 0.05)	−1.4 (−3.2; 2.4)	.108	.339	.303	
sRtot, kPa·s	0.00 (−0.07; 0.06)	0.0 (−10.7; 10.0)	.898	0.02 (−0.07; 0.08)	2.7 (−7.9; 13.8)	.398	.257	.237	log
VA, L	−0.05 (−0.17; 0.04)	−0.9 (−2.7; 0.8)	**.005****	−0.02 (−0.14; 0.03)	−0.3 (−2.2; 0.6)	**.036***	.232	.321	
DLCO, mmol/min/kPa	−0.17 (−0.46; 0.10)	−2.0 (−4.8; 1.6)	**.005****	−0.18 (−0.40; 0.12)	−2.2 (−4.2; 1.6)	**.004*****	.940	.908	
DLCO/VA, mmol/min/kPa/L	−0.02 (−0.06; 0.03)	−1.0 (−3.6; 1.9)	.093	−0.01 (−0.06; 0.03)	−0.7 (−3.9; 1.7)	.099	.771	.604	
DLNO, mmol/min/kPa	−0.77 (−2.89; 0.31)	−1.9 (−5.3; 0.9)	**.003*****	−0.98 (−2.78; 0.18)	−2.5 (−5.5; 0.5)	**<.001*****	.818	.582	
DLNO/VA, mmol/min/kPa/L	−0.07 (−0.26; 0.09)	−0.8 (−3.8; 1.2)	**.033***	−0.14 (−0.30; 0.05)	−2.2 (−3.9; 0.6)	**<.001*****	.660	.154	
FeNO, ppb	0.1 (−0.7; 1.0)	0.5 (−4.0; 5.8)	.609	0.1 (−0.9; 1.2)	0.6 (−4.9; 6.8)	.745	.636	.426	log
CANO, ppb	0.1 (−0.3; 0.4)	9.9 (−19.9; 38.7)	.505	0.1 (−0.2; 0.4)	3.4 (−18.2; 40.6)	.228	.731	.749	
8-OHdG, ng/mL	−0.015 (−0.041; 0.021)	−7.2 (−20.0; 13.1)	.074	−0.003 (−0.029; 0.027)	−2.0 (−14.6; 19.7)	.988	.200	.112	log
H_2O_2, µM	0.103 (0.034; 0.318)	41.8 (14.4; 79.3)	**<.001*****	0.101 (0.055; 0.185)	36.5 (14.1; 60.5)	**<.001*****	.777	.826	log
Nasal secretions									
IL-1β, pg/mL	4.7 (−4.4; 10.4)	10.6 (−16.2; 98.3)	.083	0.4 (−10.2; 9.6)	3.4 (−18.3; 73.9)	.823	.208	.197	log
IL-5, pg/mL	0.5 (−2.3; 11.8)	4.4 (−10.6; 103.8)	.137	0.0 (−3.2; 9.1)	0.0 (−35.9; 139.4)	.223	.675	.379	log
IL-6, pg/mL	50.4 (14.9; 127.1)	96.6 (29.9; 227.6)	**<.001*****	13.8 (−3.0; 111.2)	30.8 (−1.7; 187.7)	**.001*****	**.028***	**.031***	log
IL-8, pg/mL	−90.6 (−685.2; 343.4)	−5.8 (−32.3; 28.0)	.160	34.8 (−490.4; 478.2)	1.3 (−25.7; 32.1)	.981	.697	.482	log
GM-CSF, pg/mL	4.0 (−7.5; 20.3)	29.7 (−25.1; 144.3)	0.179	−5.1 (−15.8; 9.9)	−15.3 (−55.1; 88.7)	.146	.121	.159	log
IFNγ, pg/mL	0.0 (−54.4; 197.6)	0.0 (−30.9; 163.6)	.334	0.0 (−163.1; 142.5)	0.0 (−52.1; 70.3)	.632	.352	.362	log
TNFα, pg/mL	12.5 (−32.7; 60.2)	23.2 (−48.8; 153.6)	.234	0.0 (−34.6; 28.4)	0.0 (−45.6; 69.0)	.690	.587	.557	log
Serum									
IL-1β[a], pg/mL	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	.068	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	.917	.063	.066	log
IL-5[a], pg/mL	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	.586	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	.904	.884	.831	log
IL-6, pg/mL	−2.4 (−9.1; 2.6)	−21.9 (−60.7; 33.6)	.053	−1.2 (−7.1; 3.2)	−14.3 (−63.0; 51.5)	.253	.862	.777	log
IL-8, pg/mL	−12.5 (−50.5; 10.8)	−39.0 (−73.9; 49.8)	**.020***	−2.8 (−38.3; 14.9)	−15.3 (−67.2; 57.9)	.166	.512	.317	log
GM-CSF, pg/mL	−6.5 (−36.2; 11.3)	−17.3 (−73.3; 38.3)	.058	−5.6 (−26.7; 14.8)	−13.2 (−60.5; 38.6)	.190	.715	.383	log

(Continues)

TABLE 3 (Continued)

Parameter	LLE			HLE			Difference between changes (Wilcoxon test[d])	Interaction between changes and emitter condition (ANOVA)	
	Absolute change Median (25%; 75%)	Change in % baseline Median (25%; 75%)	Wilcoxon test[c] *P* values	Absolute change Median (25%; 75%)	Change in % baseline Median (25%; 75%)	Wilcoxon test[c] *P* values	*P* values	*P* values	Log[b]
IFNγ, pg/mL	−17.5 (−91.5; 32.0)	−48.5 (−82.1; 43.7)	.069	−15.9 (−69.5; 26.6)	−23.0 (−76.0; 82.7)	.119	.593	.465	log
TNFα, pg/mL	−2.3 (−27.5; 9.8)	−9.7 (−64.2; 49.1)	.101	−1.9 (−22.2; 14.2)	−8.3 (−57.1; 61.4)	.383	.654	.701	log
ECP, µg/L	0.9 (−0.2; 2.5)	12.3 (−2.1; 39.2)	**.002****	0.9 (−0.1; 2.3)	16.4 (−0.6; 29.5)	**<.001*****	.800	.902	log
IgE, kU/L	0.3 (−0.2; 1.3)	1.4 (−1.3; 3.8)	**.008****	0.2 (−0.3; 1.1)	1.2 (−1.8; 4.1)	**.039***	.709	.435	log

[a]Most values were below the detection limit.

[b]For parameters marked in this column, $\log_{10}$-transformed values were used to examine differential effects of exposures by Wilcoxon test or repeated-measures ANOVA.

[c]Wilcoxon test on untransformed values comparing pre- versus post-values; *P< .05, **P< .01, ***P< .005. *P* values are given without correction for multiple testing. Those marked with ** or *** are low enough to indicate significant differences even after correction by a Bonferroni factor of five. The justification of this factor is given in the Statistical Analysis section.

[d]Wilcoxon test on differences between $\log_{10}$-transformed values if indicated in the 'Log' column.

P values <.05 are highlighted in bold text.

In a third factor analysis, the serum parameters IL-6, IL-8, GM-CSF, IFNγ, TNFα, ECP, and IgE were evaluated. IL-1β and IL-5 were omitted due to the high proportion of values below the detection limits. The first factor included IL-6, IL-8, GM-CSF, IFNγ, and TNFα, and the second, ECP and IgE. The first factor showed a significant difference when comparing pre- and post-results for LLE (*P*<.05) but not HLE. The second factor showed changes after both exposures (*P*<.005 each), but there were no differences between HLE and LLE (Table 5).

3.3.6 | Participant subgroups

ANOVAs including the three participant groups as additional category did not indicate that the pre-post differences systematically varied between groups. The only exceptions were sRtot (*P*<.05), with an increase in healthy participants as opposed to a decrease in the two other groups, and 8-OHdG (*P*<.005) with an analogous pattern. Furthermore, interactions between groups and emitter condition were found for nasal GM-CSF (*P*<.05), with generally higher values in the S group at the HLE day. An analogous pattern occurred for nasal TNFα (*P*<.01). For serum IL-5 (*P*<.05), there were generally higher values in the asthma group on the HLE day. No triple interactions between participant groups, pre-post changes and emitter condition, and no interactions with participant groups in factor scores were found. A similar picture was observed in ANOVAs including BHR as alternative category across groups. Significant interactions of BHR with pre-post responses were found for serum IL-5 and IL-8 as well as nasal IL-1β and IL-6. The observed effects regarding IL-1β and IL-6 were in parallel to those observed in the factor scores. No significant interactions of BHR with emitter condition or triple interactions between BHR, pre-post changes, and emitter condition occurred.

4 | DISCUSSION

The present study investigated physiological and biochemical responses after controlled exposures to laser printer emissions in three groups of subjects. The two exposures were performed in a randomized, cross-over, single-blinded fashion, and the number concentrations of nanoparticles were either at background level or very high (10^5 particles/cm^3). There were statistically significant, although small, changes in a number of parameters, but none of them showed a consistent difference between HLE and LLE, except for nasal IL-6. The observed changes were similar for both exposures and might be due to circadian effects or the repeated procedures performed for measurement. Taking into account multiple testing via a tentative Bonferroni correction factor of 5, a number of pre-post effects remained (see Table 3); however, the only consistent difference between HLE and LLE regarding nasal IL-6 became non-significant. To find more global patterns of changes, we additionally employed factor analysis (principal components) for condensing the information contained in the different parameters and their relationship. Using this approach, the correlation structure within and between the different sets of parameters became clear, but no additional

TABLE 4 (a) Factor analysis of lung function parameters: factor loadings. The three factors account for 84.1% of the original variance. (b) Factor analysis of nasal secretion parameters: factor loadings. The two factors account for 79.1% of the original variance. (c) Factor analysis of blood-derived parameters: factor loadings. The two factors account for 76.8% of the original variance.

(a)			
Parameter	**Lung factor 1**	**Lung factor 2**	**Lung factor 3**
FEV_1	0.122	0.247	0.880
FEV_1/FVC	0.258	0.888	−0.043
ITGV	−0.184	−0.249	0.876
$\log_{10}$sRaw	0.393	−0.800	−0.062
DLCO/VA	0.897	−0.051	0.015
DLNO/VA	0.931	0.039	−0.066

(b)		
Parameter	**Nasal factor 1**	**Nasal factor 2**
$\log_{10}$IL-1β	0.149	0.910
$\log_{10}$IL-5	0.850	0.233
$\log_{10}$IL-6	0.168	0.805
$\log_{10}$IL-8	−0.219	0.821
$\log_{10}$GM-CSF	0.904	0.152
$\log_{10}$IFNγ	0.917	−0.127
$\log_{10}$TNFα	0.903	−0.073

(c)		
Parameter	**Blood factor 1**	**Blood factor 2**
$\log_{10}$IL-6	0.929	−0.025
$\log_{10}$IL-8	0.926	−0.010
$\log_{10}$GM-CSF	0.917	0.004
$\log_{10}$IFNγ	0.936	−0.035
$\log_{10}$TNFα	0.870	−0.078
$\log_{10}$ECP	−0.096	0.743
$\log_{10}$IgE	0.049	0.783

hints on differences between exposures were found. Moreover, the subjects with mild asthma and those with a self-reported history of LPD-associated symptoms showed no consistent differences in their responses. The findings indicate that a short-term exposure to very high levels of LPD emissions does not elicit marked changes in lung function parameters or biochemical markers that could be associated with the symptoms typically reported after exposure to such emissions.

The fact that spirometry and body plethysmography did not hint on statistically or clinically significant differential effects was not unexpected. These parameters had been included primarily with the intention to check the comparability of the functional status between exposures. Of more interest seemed the changes in the transfer factors for CO and NO. After correction for COHb and alveolar volume, there were no significant alterations and differences between exposures in TLCO and thus no hints on changes in pulmonary capillary blood volume, as a major determinant of CO uptake in subjects without parenchymal disorders.

The NO transfer factor is much less dependent on the amount of hemoglobin in the lung than CO and more suited to detect limitations of diffusion in the proper sense. There were significant changes in this measure, and the effect after HLE seemed to be larger than after LLE, although the difference between exposures did not reach statistical significance. It is tempting to interpret this as a tendency toward a very small limitation of gas uptake. Mechanisms for this remain speculative, but various possibilities could be imagined, for example, a fluid imbalance in the alveolar region, or the formation of VOC layers arising from the inhaled LPD particles, or interactions with the structures determining gas transfer from the alveoli into the blood. In this case, one might also have expected a reduction in alveolar NO (CANO) which probably takes the reverse way from the blood into the exhaled air. Despite many efforts put into the standardization and quality control, there remain doubts regarding the validity of the estimates that are derived via an idealized mathematical model; modifications of the computational procedure using FeNO were not further considered as there were also no changes in FeNO. Due to

TABLE 5 Overview of low-level (LLE) and high-level exposure (HLE) effects on factors derived from factor analysis

	LLE	HLE		
Parameter	Pre vs. Post (Wilcoxon test) *P* values	Pre vs. Post (Wilcoxon test) *P* values	Difference between changes (Wilcoxon test) *P* values	Interaction between changes and emitter condition (ANOVA) *P* values
Lung factor 1	.088	**.043**	.948	.576
Lung factor 2	.227	.339	.771	.502
Lung factor 3	.771	.117	.227	.219
Nasal factor 1	.110	.612	.615	.748
Nasal factor 2	**.001**	**.019**	.400	.335
Blood factor 1	**.026**	.104	.735	.582
Blood factor 2	**<.001**	**<.001**	.767	.712

P values <.05 are highlighted in bold text.

these difficulties, the lack of changes in CANO does not necessarily contradict the interpretation of the effects observed for TLNO. The assumption of a diffusion limitation indicated by TLNO is supported by the results of a methodological pre-study that have been reported in short form.[34] It showed that inhalation of hypertonic saline solution led to a reduction in TLNO, whereas the reduction in TLCO was smaller and not statistically significant. Such a result would be explained if the hypertonic saline aerosols influence the fluid balance in the alveolar region. We therefore consider the tendency toward a reduction in NO diffusing capacity after HLE as a hint that the high level of inhaled nanoparticles might have elicited a small and clinically irrelevant but detectable alteration in the alveolar region, most likely due to a change in fluid layers. It would be interesting to verify the presence of such effects by independent methods, for example, MRI methods averaging over the whole lung.

We used state-of-the-art methods to measure NO and H_2O_2 in exhaled air. Exhaled NO, both the standard bronchial NO (FeNO) and the alveolar NO (CANO), did not show any significant changes. Such differences or effects would be expected if inflammatory responses associated with eosinophils would have occurred, or—more likely—changes affecting gas transport in the surface of the airways or the lung periphery. This could be due to fluid imbalance or production of oxidants scavenging part of the produced NO. Exhaled H_2O_2, which we considered as marker of respiratory oxidative stress, was elevated after both exposures but without a difference between the changes. This finding might have been either result of circadian variation or a side effect of the assessments performed prior to the second H_2O_2 measurement. It is unlikely that changes in ambient air levels were responsible, as we used an optimized setup largely removing H_2O_2 from inhaled ambient air.[33] In accordance with that, serum 8-OHdG, which we considered as a potential marker of systemic oxidative stress, was not affected by exposures.

Inflammatory and biochemical markers were also assessed in blood and nasal secretions. There were no consistent changes in cytokines and chemokines assessed in serum samples. The changes in ECP were probably due to circadian effects and not significantly different between exposures; the same was true for IgE levels that had been measured to characterize the participants. In contrast to serum, some effects were observed in nasal secretions, particularly regarding IL-6. This marker was elevated after both exposures, and the change was more pronounced after LLE than HLE. It is difficult to interpret this pattern. Potential dilution effects of nasal secretions in repeated measurements would also be expected for the other markers, in contrast to the data. A significant inhibition of IL-6 production by high particle emissions is rather unlikely, in particular as Khatri et al. observed an increase in nasal IL-6 levels after exposure of nine healthy participants in a photocopy center.[16] These authors also found changes in other cytokines that were unaffected in our exposures, as well as an increase in 8-OHdG in urine samples. Besides methodological factors, the differences in the duration of exposures and the time points of measurements could contribute to the difference of findings. Moreover, in their study, the photocopying activity was not standardized and control measurements seemed to be performed in a different location.

At present, our study is the only controlled exposure study to standardized LPD emissions in human subjects. There are observational studies involving subjects working in copying centers and control subjects outside such centers.[17,35] These studies may be suitable to detect long-term effects but certainly depend on the control population and on confounders including social factors. For example, the study by Karimi et al. on effects on lung function showed a higher percentage of smokers in the exposed compared to the control group. The study by Elango et al. showed very high background levels of air pollution, as indicated by PM_{10} and $PM_{2.5}$, which renders it difficult to separate the effects attributed to LPD emissions from those of ambient air pollution. Moreover, Karimi et al. reported changes in spirometric parameters, whereas Elango et al. found no such changes but changes in several markers of inflammation. It is unclear whether this difference is due to uncontrolled confounders. Our study was a short-term experimental study. Against the background of observations from other studies, the question of possible chronic and/or long-term effects of LPD exposure remains open. The host of potential confounders and the multitude of effects attributed to LPD exposure, which was also reflected in the complaints reported in the S group, render it difficult to predict long-term or chronic effects in terms of well-defined diseases. An important step in further research would be a data set of realistic long-term estimates of LPD exposure levels.[36]

Short-term effects of LPD emissions have also been assessed in an observational study in six subjects using a spectrum of lung function parameters and inflammatory markers.[37] There were alterations regarding oxidative stress, but the results are difficult to generalize due to the small sample size. Exposure levels went up to 100 000 cm^{-3} for 30 minutes, whereas we exposed 52 subjects to a constant level of 100 000 cm^{-3} for about 75 minutes. It thus seems unlikely that we missed acute effects regarding the state of the respiratory system, or systemic effects. In a study investigating a broad panel of markers in 69 subjects exposed to LPD emissions at the workplace,[38] the subjects reported symptoms but the objective measurements were inconclusive and there were no proper controls. All of the studies mentioned provide hints on possible effects but are limited by a lack of control and standardization. At the first sight, the occurrence of adverse effects of LPD emissions on human health seems conceivable as particulate matter of ambient air pollution is known to exert such effects. On the other hand, it has been estimated that the potential health impact of LPD exposure is less than that of ambient air pollution.[36]

Compared to previous studies, the present one was highly standardized and included adequate control exposures as well as a relatively large sample size. It also comprised a broad panel of markers, the assessment of which had been optimized in pre-studies. Moreover, we not only included subjects who reported symptoms from exposure to LPD emissions but also subjects with mild asthma who could be considered as most susceptible regarding objective respiratory responses. In none of the parameters assessed, there were consistent differences between the responses of the three groups. We therefore were able to maximize the power of the study by pooling over all subjects, and the resulting total number (n=52) was probably sufficient to detect effects of a clinically relevant magnitude.

An experimental study necessarily has limitations regarding the duration and number of exposures as well as the observation period after exposures. One limitation of our study is that it comprised a single, although very high, short-term exposure and covered effects occurring within 2 h after exposure. Typically, however, the symptoms reported after LPD exposure are of acute nature and largely refer to the respiratory tract. Functional and biochemical alterations of the upper and lower airways seemed to be covered by the parameters assessed in our study. Further parameters might have been desirable but high precision measurements take time, often require high cooperation by the participants, and might interfere with each other; for example, sputum induction or the assessment of bronchial hyperreactivity might not be compatible with other measurements performed at about the same time. The high degree of precision achieved in our study was reflected in the fact that even tiny differences could be demonstrated as statistically significant.

We therefore consider it reasonable that the time frame and parameter panel chosen for this study conferred a high probability to detect differential effects between HLE and LLE. Naturally, one single exposure is not capable of detecting long-term or chronic effects that require repeated exposures. Such effects are conceivable and have been demonstrated, for example, for inhaled ozone and bronchial allergen responsiveness.[39] Due to logistic reasons, it was not possible to extend the post-exposure observation period.

5 | CONCLUSIONS

The present experimental cross-over study included participants who reported symptoms from exposure to LPD emissions and participants with mild asthma, in addition to control subjects. The former two groups can be considered to be especially susceptible to acute effects of LPD exposure. Exposure levels were either very high (HLE) or very low (LLE) and maintained over more than 1h. The assessments comprised standard and advanced lung function measurements, as well as biomarkers from blood and upper and lower airways. There were no consistent differences between the effects after HLE versus LLE. Our findings do not exclude potential effects of repeated or longer exposures, or effects in parameters not assessed. Irrespective of these limitations, they do not indicate the occurrence of acute, clinically relevant alterations after a single high-level exposure to LPD emissions and restrict the range of objectively testable effects that can plausibly be claimed.

ACKNOWLEDGEMENT

We thank all participants for their patience and commitment in performing the study. In particular, we gratefully acknowledge the efforts taken by the participants who had reported previous complaints from LPD exposure. This work was supported by the German Statutory Accident Insurance (FP294). The funders did not intervene with the carrying out of the study, data collection and analysis, decision to publish, or preparation and content of the manuscript.

REFERENCES

1. Destaillats H, Maddalena RL, Singer BC, et al. Indoor pollutants emitted by office equipment: a review of reported data and information needs. *Atmos Environ*. 2008;42:1371–1388.
2. Kagi N, Fujii S, Horiba Y, et al. Indoor air quality for chemical and ultrafine particle contaminants from printers. *Build Environ*. 2007;42:1949–1954.
3. Lee C-W, Hsu D-J. Measurements of fine and ultrafine particles formation in photocopy centers in Taiwan. *Atmos Environ*. 2007;41:6598–6609.
4. Schripp T, Wensing M, Uhde E, et al. Evaluation of ultrafine particle emissions from laser printers using emission test chambers. *Environ Sci Technol*. 2008;42:4338–4343.
5. Seeger S. Time- and size-resolved characterisation of particle emissions from office machines. Healthy Buildings Conference, 2006: Lisbon. 2006:447–450.
6. He C, Morawska L, Taplin L. Particle emission characteristics of office printers. *Environ Sci Technol*. 2007;41:6039–6045.
7. Kumar S, Verma MK, Srivastava AK. Ultrafine particles in urban ambient air and their health perspectives. *Rev Environ Health*. 2013;28:117–128.
8. He C. Contribution from indoor sources to particle number and mass concentrations in residential houses. *Atmos Environ*. 2004;38:3405–3415.
9. Wallace LA, Emmerich SJ, Howard-Reed C. Source strengths of ultrafine and fine particles due to cooking with a gas stove. *Environ Sci Technol*. 2004;38:2304–2311.
10. Géhin E, Ramalho O, Kirchner S. Size distribution and emission rate measurement of fine and ultrafine particle from indoor human activities. *Atmos Environ*. 2008;42:8341–8352.
11. Matson U. Indoor and outdoor concentrations of ultrafine particles in some Scandinavian rural and urban areas. *Sci Total Environ*. 2005;343:169–176.
12. Jensen M, Roed-Petersen J. Itching erythema among post office workers caused by a photocopying machine with wet toner. *Contact Dermatitis*. 1979;5:389–391.
13. Guidotti TL. Angiitis and photocopier fumes. *Ann Intern Med*. 1983;99:570.
14. Skoner DP, Hodgson MJ, Doyle WJ. Laser-printer rhinitis. *N Engl J Med*. 1990;322:1323.
15. Gallardo M, Romero P, Sanchez-Quevedo MC, Lopez-Caballero JJ. Siderosilicosis due to photocopier toner dust. *Lancet*. 1994;344:412–413.
16. Khatri M, Bello D, Gaines P, et al. Nanoparticles from photocopiers induce oxidative stress and upper respiratory tract inflammation in healthy volunteers. *Nanotoxicology*. 2013;7:1014–1027.
17. Karimi A, Eslamizad S, Mostafaee M, et al. Restrictive pattern of pulmonary symptoms among photocopy and printing workers: a retrospective cohort study. *J Research Health Sci*. 2016;16:81–84.
18. Ikegami K, Hasegawa M, Ando H, et al. A cohort study of the acute and chronic respiratory effects of toner exposure among handlers: a longitudinal analyses from 2004 to 2013. *Ind Health*. 2016;54:448–459.
19. Kitamura H, Terunuma N, Kurosaki S, et al. A cohort study on self-reported respiratory symptoms of toner-handling workers: cross-sectional and longitudinal analysis from 2003 to 2008. *Biomed Res Int*. 2014;2014:826757.
20. Kitamura H, Terunuma N, Kurosaki S, et al. A cohort study of toner-handling workers on inflammatory, allergic, and oxidative stress markers: cross-sectional and longitudinal analyses from 2003 to 2008. *Hum Exp Toxicol*. 2015;34:337–344.
21. Barthel M, Pedan V, Hahn O, et al. XRF-analysis of fine and ultrafine particles emitted from laser printing devices. *Environ Sci Technol*. 2011;45:7819–7825.
22. Morawska L, He C, Johnson G, et al. An investigation into the characteristics and formation mechanisms of particles originating from the operation of laser printers. *Environ Sci Technol*. 2009;43:1015–1022.
23. Pirela S, Molina R, Watson C, et al. Effects of copy center particles on the lungs: a toxicological characterization using a Balb/c mouse model. *Inhal Toxicol*. 2013;25:498–508.
24. Sisler JD, Pirela SV, Friend S, et al. Small airway epithelial cells exposure to printer-emitted engineered nanoparticles induces cellular effects on human microvascular endothelial cells in an alveolar-capillary co-culture model. *Nanotoxicology*. 2015;9:769–779.
25. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338.
26. Criée C, Berdel D, Heise D, et al. *Empfehlungen zur Ganzkörperplethysmographie*. München, Orlando: Dustri-Verlag Dr. Karl Feistle; 2009.
27. Klein G, Baur X, Becker R, et al. Leitlinien für die Durchführung bronchialer Provokationstests mit pharmakologischen Substanzen. *Pneumologie*. 1998;52:214–220.
28. Cotes JE, Chinn DJ, Quanjer PH, et al. Standardization of the measurement of transfer factor (diffusing capacity). Report working party standardization of lung function tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl*. 1993;16:41–52.
29. Hughes JM, Van Der Lee I. The TL, NO/TL, CO ratio in pulmonary function test interpretation. *Eur Respir J*. 2013;41:453–461.
30. Graham BL, Mink JT, Cotton DJ. Effects of increasing carboxyhemoglobin on the single breath carbon monoxide diffusing capacity. *Am J Respir Crit Care Med*. 2002;165:1504–1510.
31. American Thoracic Society and European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005;171:912–930.
32. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol*. 1985;1998:653–666.
33. Peters S, Kronseder A, Karrasch S, et al. Hydrogen peroxide in exhaled air: a source of error, a paradox and its resolution. *ERJ Open Res*. 2016;2:00052–2015.
34. Ehret M, Karrasch S, Kronseder A, et al. Effects of inhaled saline and oxygen on noninvasive markers of airway and lung function. *Eur Respir J*. 2012;40:P3495.
35. Elango N, Kasi V, Vembhu B, Poornima JG. Chronic exposure to emissions from photocopiers in copy shops causes oxidative stress and systematic inflammation among photocopier operators in India. *Environ Health*. 2013;12:78.
36. Hänninen O, Brüske-Hohlfeld I, Loh M, et al. Occupational and consumer risk estimates for nanoparticles emitted by laser printers. *J Nanopart Res*. 2009;12:91–99.
37. Mersch-Sundermann V, Ebner W, Hurraß J, Gminski R. Effect of laser printer emission exposure on human health – Investigation of six patients including ESR measurements. *Toxicol Lett*. 2011;205:S223.
38. Mersch-Sundermann V (2008) Evaluierung möglicher Beziehungen zwischen Emissionen aus Büromaschinen, insbesondere aus Fotokopieren und Laserdruckern, und Gesundheitsbeeinträchtigungen bzw. Gesundheitsschäden bei exponierten Büroangestellten. 2008. UFO-Plan FKZ 705 62 449, BFR.
39. Holz O, Mücke M, Paasch K, et al. Repeated ozone exposures enhance bronchial allergen responses in subjects with rhinitis or asthma. *Clin Exp Allergy*. 2002;32:681–689.

How to cite this article: Karrasch S, Simon M, Herbig B, et al. Health effects of laser printer emissions: a controlled exposure study. *Indoor Air*. 2017;00:1-13. doi: 10.1111/ina.12366